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THE IN VITRO INFLUENCE OF THE BURROWING POLYCHAETE NEREIS DIVERSICOLOR
ON THE FATE OF PETROLEUM HYDROCARBONS IN MARINE SEDIMENTS.

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Abstract : The *in vitro* fate of the saturated hydrocarbon fraction (SF) of Arabian Light crude oil has been studied in PVC cores filled with a coastal marine sediment defaunated by sieving. Experiments were conducted in absence or presence of polychaetes *Nereis diversicolor*. The luminophore tracer technique was used to quantify the mixing of sediment by worms. Presence of crude oil reduced the building of burrows by polychaetes. This work demonstrates the ability of infaunal organisms to stimulate both downward and outward transfers of hydrocarbons from sediment reservoirs. In non-bioturbated sediment hydrocarbons were confined to the sediment surface. Introduction of polychaetes in sediment (1) induced the burying of SF in sediment (2.5 % and 13.5 % of the initial surface input after 15 and 45 days, respectively); (2) enhanced the exportation of SF in the overlying water (plus 59 % and 23.5 % compared to defaunated control sediment after 15 and 45 days, respectively). Buried hydrocarbons were submitted to biodegradation, from 2 cm to 10 cm depth in polychaete burrows, after 45 days.

Bioturbation plays an important role in organic matter decomposition by increasing the water-sediment interface¹ and irrigating sediment. Most burrow constructors maintain contact with the overlying water by

ventilating water through their burrow systems^{2,3} and thus increase the transport of ions and gases (e.g. oxygen) over the water-sediment interface^{4,5,6}.

In the same way, burrowing activity may also alter pollutant concentration in sediments. Through the activities of burrowing populations, deposited pollutants become buried. This may result in long-lasting negative effects on the marine ecosystem. On the other hand, buried components may be released more quickly than can be explained by normal molecular diffusion coefficients^{7,8}.

Among the pollutants, numerous studies have investigated the fate of petroleum hydrocarbons in marine sediments^{9,10,11,12}. However, only a few works have taken into account the influence of burrowing macrofauna activity on the fate of hydrocarbons in the sediments^{13,14,15}.

In estuarine ecosystem, the infaunal polychaetes from the Nereidae family are known to generate significant changes in the properties of the sediment-water interface^{1,16,17}. Our purpose was to study the influence of burrowing activity on the fate of hydrocarbons in relation with the mixing of sediment quantified by the luminophore tracer technique^{18,19}. We therefore decided to carry out an *in vitro* experiment with the polychaete *Nereis diversicolor* whose burrowing activity results in both the mixing and the irrigation of different layers of the sediment²⁰.

EXPERIMENTAL

- Collection of polychaetes and sediment : During July 1991, sediment and polychaetes were collected by digging in the Carteau cove (Gulf of Fos, France). The sampling site has already been described¹⁸. An undisturbed sediment core was sampled using a PVC corer (i. d. 7.3 cm) of 30 cm length to examine sediment characteristics before the start of the experiment (**A**, figure 1). In the laboratory, the sediment was wet-sieved through a 1 mm sieve to remove macrofauna and thoroughly homogenized by gloved hands (**B**). Then, eight cores (i. d. 7.3 cm; 30 cm length) were filled to a depth of 20 cm with macrofauna-free sediment and placed in an aquarium thermostated at 17° C under a LD 12:12 cycle (**C**). By sieving of the sediment, *Nereis diversicolor* individuals were obtained (**D**). The polychaetes were acclimatized to experimental conditions, 17°C and 30 ‰ S, for 6 d in a recirculating seawater aquarium partially filled with natural sediment before being introduced into cores. Then, six weighed *N. diversicolor* (0.64 to 0.81 g wet weight) were added (**E**) to 6 cores (~ 1433 indiv.m⁻² ; ~ 1 wt kg.m⁻²). Several animals that did not burrow within 12 hours were removed and replaced with fresh ones of same weight.

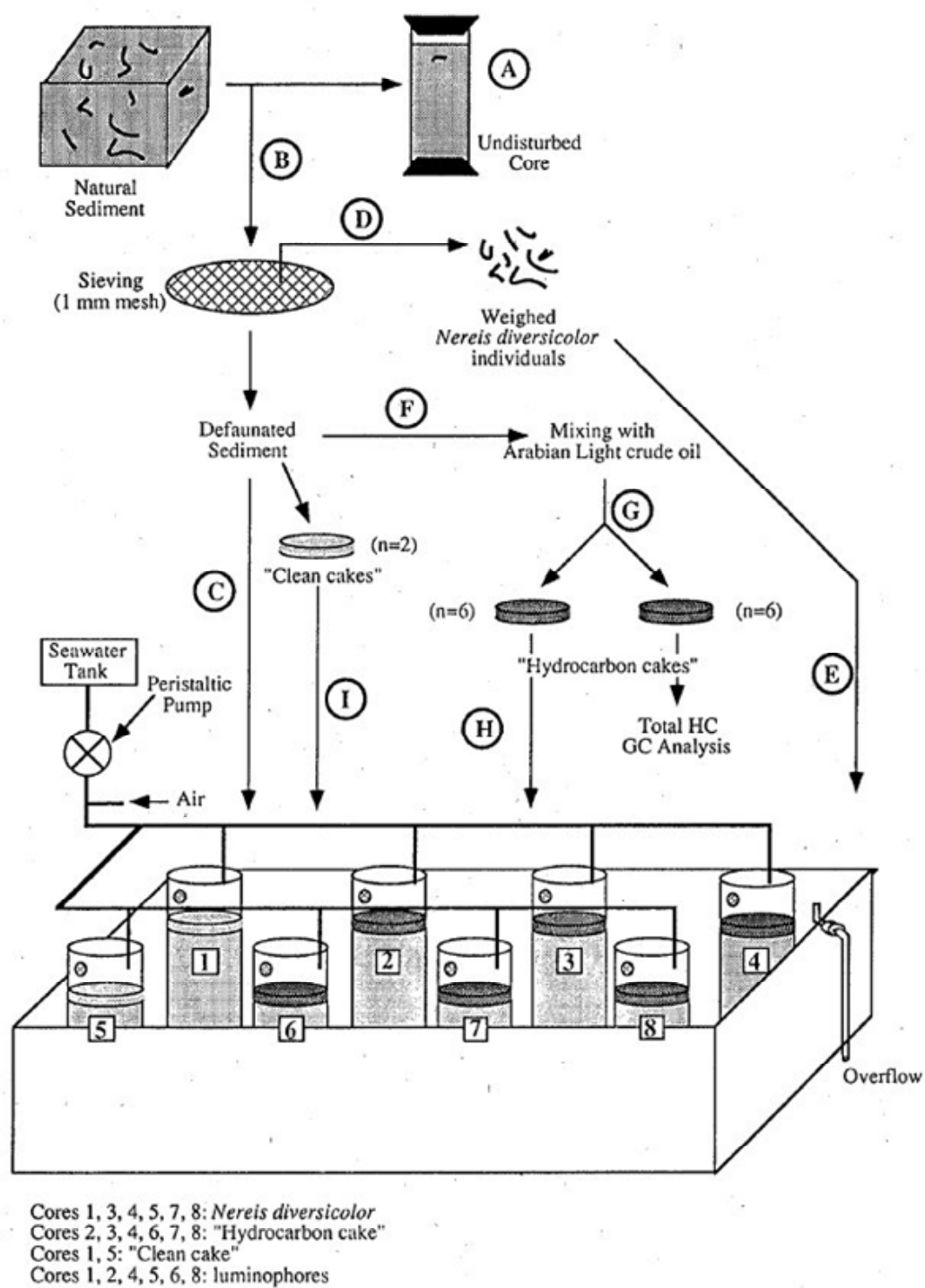


Figure 1: Experimental procedure. The different steps (letters in brackets) are described in detail in text.

- Continuous flow system : A peristaltic pump system supplied sediments with seawater. Four multiple-head peristaltic pumps (Cole-Parmer, Chicago, IL) controled filtered seawater flow (0.6 ml min^{-1}) to the cores from two 50-litre thermostated tanks. Overlying the sediment of each core was approximatively 0.3 litre of seawater which was kept aerated by a small aquarium pump attached to an airstone. The evacuation of water was allowed through a 1 mm perforation bored 3 cm from the core top.

- Petroleum hydrocarbons : A mixture of Arabian Light crude oil and sediment (**F**) was used to contaminate cores²¹. Then, 12 "hydrocarbon cakes" were made by filling moulds (i. d.: 10 cm, 1 cm thick), and frozen (**G**). GC analysis showed that the concentration of the saturated fraction of hydrocarbons (SF) in each cake was $3215 \pm 25 \text{ mg SF. kg}^{-1} \text{ dry sediment}$ (mean \pm CI, $n=6$). Frozen hydrocarbon cakes were deposited at the sediment surface of six cores (**H**). The two other cores received a "clean" cake made with sieved sediment to ensure standard sediment height (**I**).

After 15 and 45 d, sediment cores were sectioned to 2 cm thick segments from the top to 10 cm, where the analyses were performed.

The extraction and fractionation of petroleum hydrocarbons from the sediment, and the saturated fraction analysis were carried out as described by Bertrand et al.²².

- Analysis of sediment mixing : The mixing of sediment was quantified with the luminophore tracer technique^{18,19}. Two size fractions of luminophores, 40-60 μm diameter (red) and 150-200 μm diameter (yellow) were used. Then, for six cores, a mixture of the two luminophore fractions (0.5 g per fraction) was added at the cake surface. When removed, each segment of sediment was dried at 70°C for a week , carefully mixed to homogeneize sediment and luminophores, and sieved through a 500 μm mesh. For each segment, 3 subsamples of 0.25 g were taken. The luminophores counts were then conducted under an ultra-violet light source.

RESULTS AND DISCUSSION

There is a significant correlation between the surface of the burrow walls of *N. diversicolor* and the quantity of luminophores at each level, except the first layer (0-2 cm) because luminophores are deposited in excess at the surface²³. Table I presents the distribution of luminophores in control defaunated sediment, and experimental sediments (with 6 *Nereis diversicolor*) contaminated and not contaminated, after 15 and 45 d. It is first shown that the building of burrows by the worms was inhibited in the presence of crude oil. The quantities of

luminophores buried in contaminated sediment were on average 77 % lower than those found in non-contaminated sediment, after 15 days.

Table I

Distribution of luminophores in control defaunated sediment and experimental sediments (with 6 *Nereis diversicolor*). NC, non-contaminated sediment; AL, Arabian Light crude oil contaminated sediment. Values are expressed in milligrams for an 1 g initial luminophore input.

Depth	15 d			45 d		
	Control sediment	Inhabited	sediment	Control sediment	Inhabited	sediment
		NC	AL		NC	AL
0-2 cm	760.1	871.5	629.3	720.2	503.3	490.4
2-4 cm	0.0	62.6	36.4	0.0	83.4	79.8
4-6 cm	0.0	77.4	16.7	0.0	52.0	48.5
6-8 cm	0.0	55.6	1.5	0.0	83.0	25.9
8-10 cm	0.0	39.3	2.3	0.0	64.2	37.8

After 45 days, differences between "clean" and contaminated sediments were about 54 %, and could only be seen below 6-cm depth. Research has already shown that the 5-day sediment reworking rate of *Arenicola marina* was about 70 % reduced in presence of a surface sediment oil concentration of 2086 mg. kg⁻¹ dry sediment²⁴, and when natural populations were sprayed with oil²⁵ at a rate of 0.2 l. m⁻².

Experiments were carried out on a artificially contaminated sediment. Before contamination, the saturated hydrocarbon composition of natural sediment was essentially due to biogenic hydrocarbons (figure 2). Arabian Light crude oil was chosen as source of hydrocarbons because there was no analogy between its *n*-alcanes distribution of SF (figure 3) and that of the natural sediment one.

In control cores, hydrocarbons were kept at the sediment surface (Table II). In inhabited sediments, the presence of polychaetes induced the burying of the crude oil (Table II and figure 4). Gravimetric results have also shown that the distribution of added hydrocarbons in experimental (with polychaetes) sediment was not

equal with depth and time. After 15 d, only very low quantities of crude oil were detected (only by gas-chromatography) under 6 cm depth. After 45d, crude oil was detected throughout the whole sediment depth.

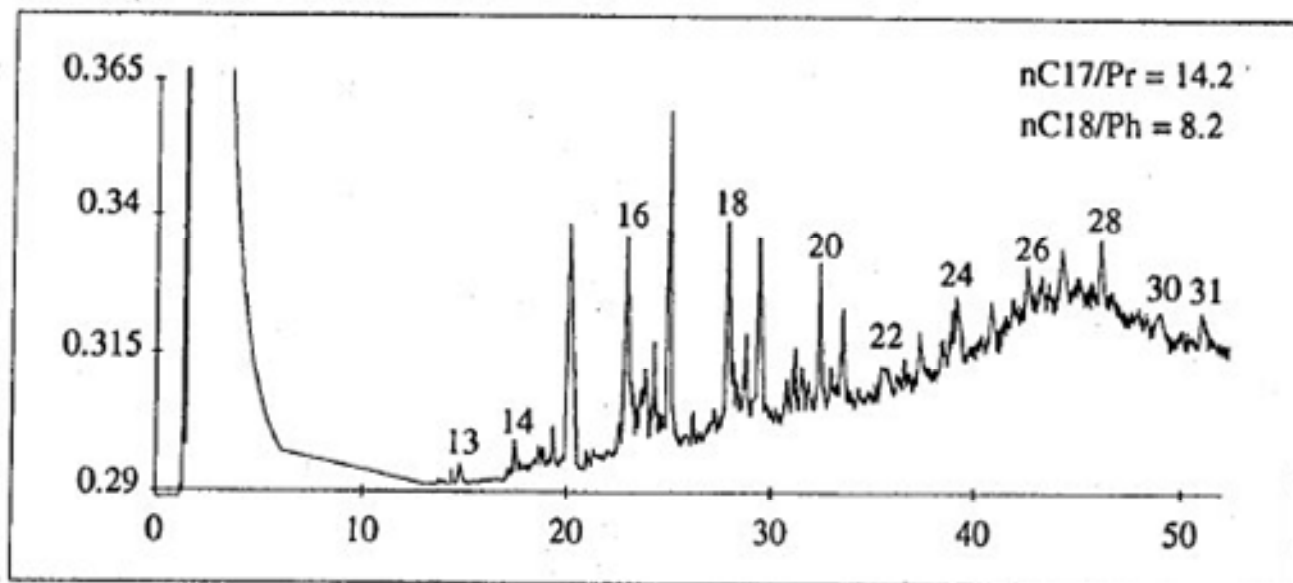


Figure 2: GC-chromatogram of the saturated hydrocarbon fraction extracted from the natural sediment; Pr: pristane; Ph: phytane; *n*-alkanes (numbers from 13 to 31) are labelled with the number of carbons in the molecule.

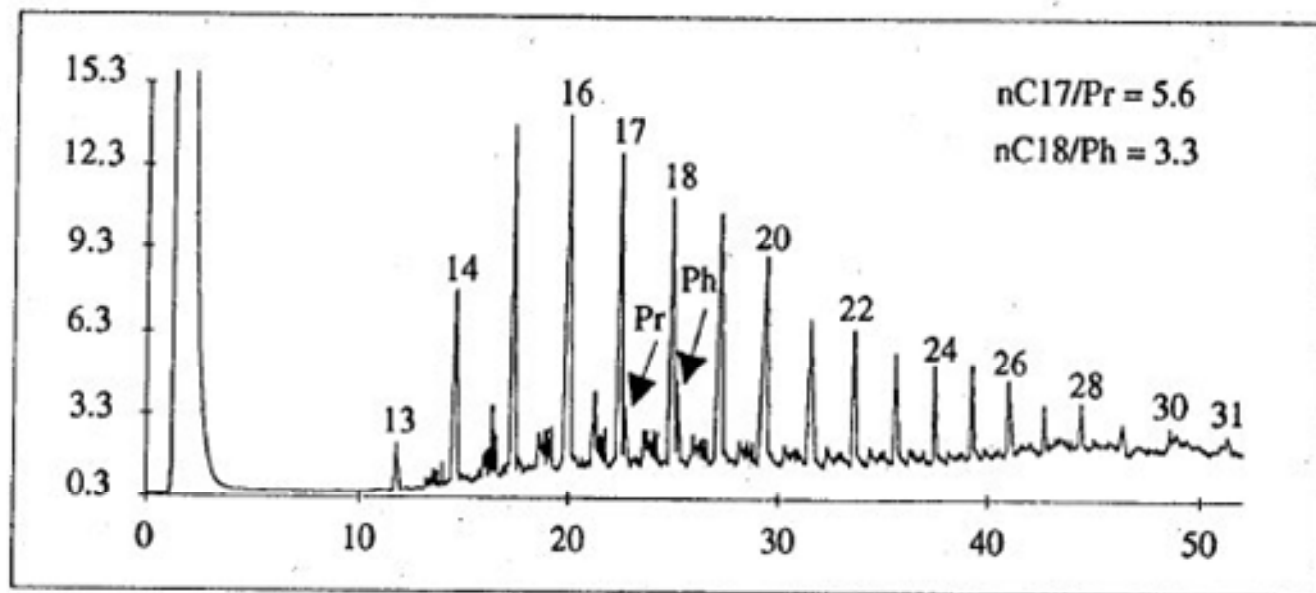


Figure 3: GC-chromatogram of the saturated hydrocarbon fraction extracted from the "hydrocarbon cake" used to contaminate sediment. Pr: pristane; Ph: phytane; *n*-alkanes (numbers from 13 to 31) are labelled with the number of carbons in the molecule.

If we compare the concentrations of SF and the quantities of luminophores in the experimental sediment which have been contaminated by crude oil, no correlation is apparent. However, it is clearly demonstrated that when

the surface of burrows is very low (e.g. layers 6-8 cm and 8-10 cm, after 15 d) (Table I), only traces of crude oil are detectable (Table II). We conclude that the burying of hydrocarbons is only qualitatively related with the building of burrows by the polychaetes.

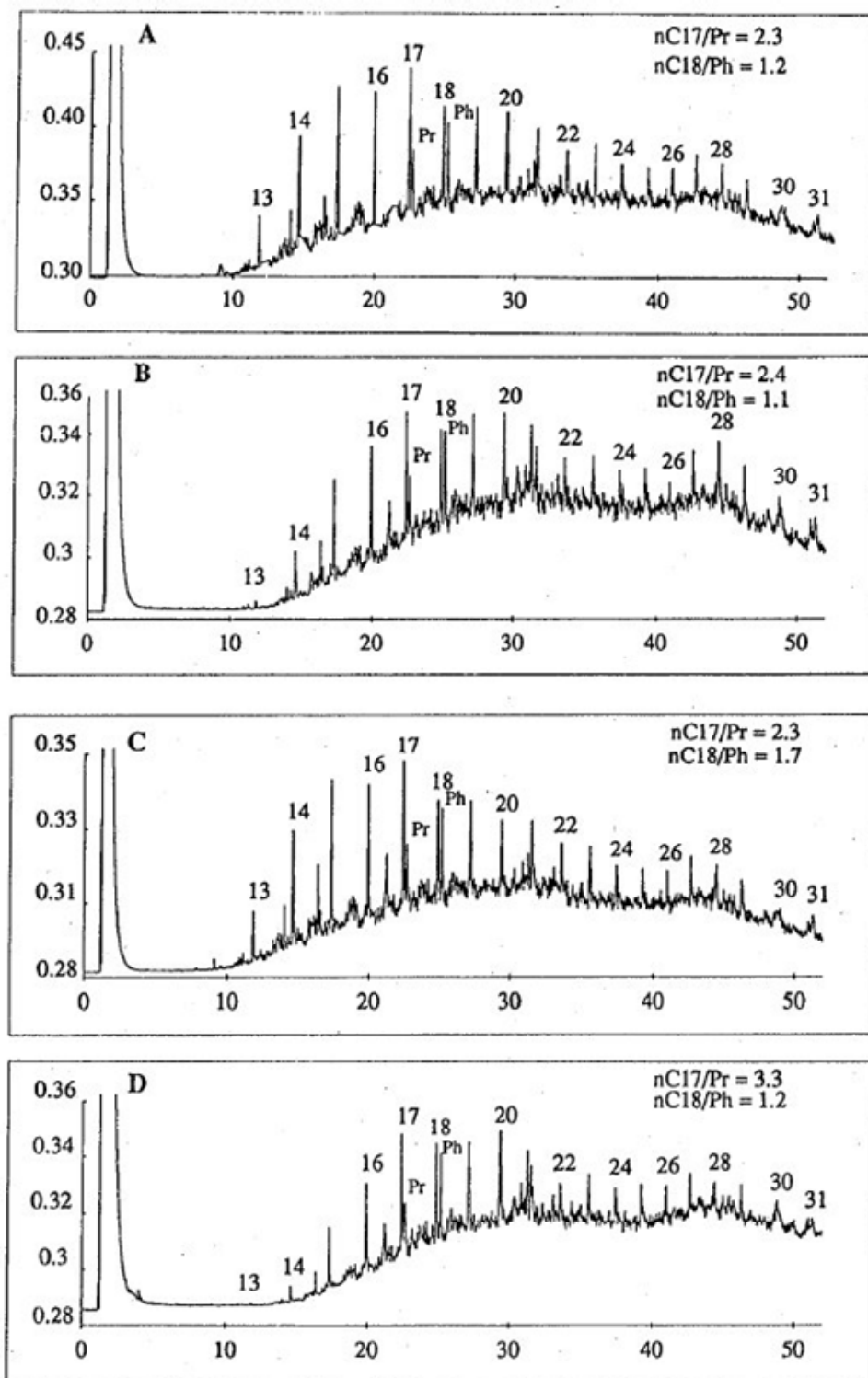


Figure 4: GC-chromatograms of the saturated hydrocarbon fraction extracted from the experimental sediment with 6 *Nereis diversicolor*, after 45 d. A: 2-4 cm depth; B: 4-6 cm depth; C: 6-8 cm depth; D: 8-10 cm depth; Pr: pristane; Ph: phytane; *n*-alkanes (numbers from 13 to 31) are labelled with the number of carbons in the molecule.

Table II

Concentrations of SF in the control (without *Nereis diversicolor*) and experimental sediment (with 6 *Nereis diversicolor*), after 15 and 45 d. Concentrations are expressed in mg. kg⁻¹ dry sediment.

Depth (cm)	15 d		45 d	
	C	I	C	I
0-2	1543	555	761	152
2-4	26	53	29	100
4-6	21	35	18	39
6-8	17	16	15	36
8-10	20	20	19	40

SF, saturated hydrocarbon fraction; C, control defaunated sediment; I, inhabited sediment.

Results can be presented as the percentages, in the water-sediment interface, of luminophores and SF compared to quantities initially deposited. Studying the transfers of luminophores (figure 5) seems to suggest that the presence of polychaetes did not have any influence on their exportation in the water column (from 24 % to 28 % in control sediment; 32 % in experimental sediment). Exportation of luminophores in the water column seems to be only due to the hydrodynamic conditions during experimentation, especially circulating water and oxygenation of water near the sediment surface. The burying of luminophores which never occurred in control sediment was respectively of 6 % (15 d) and 19 % (45 d) in experimental inhabited sediment.

Figure 6 shows the estimated hydrocarbon transfers in control and experimental sediment. After 15 d, 2.5 % of the crude oil deposited in the surface of the experimental sediment was buried, as compared to 0 % for the control sediment. The presence of the polychaetes also induced the disappearance in the water column of 63 % of the initial quantity; this exportation was only about 4 % in the control sediment. After 45 d, in control sediment,

only 47 % of the initial hydrocarbon input remained in the sediment. In experimental sediment, 13.5 % of the hydrocarbons were buried, 76.5 % exported in the water column and 10 % kept at the sediment surface. The presence of the worms stimulated the removal of hydrocarbons deposited in surface (plus 23.5 % compared to the control sediment).

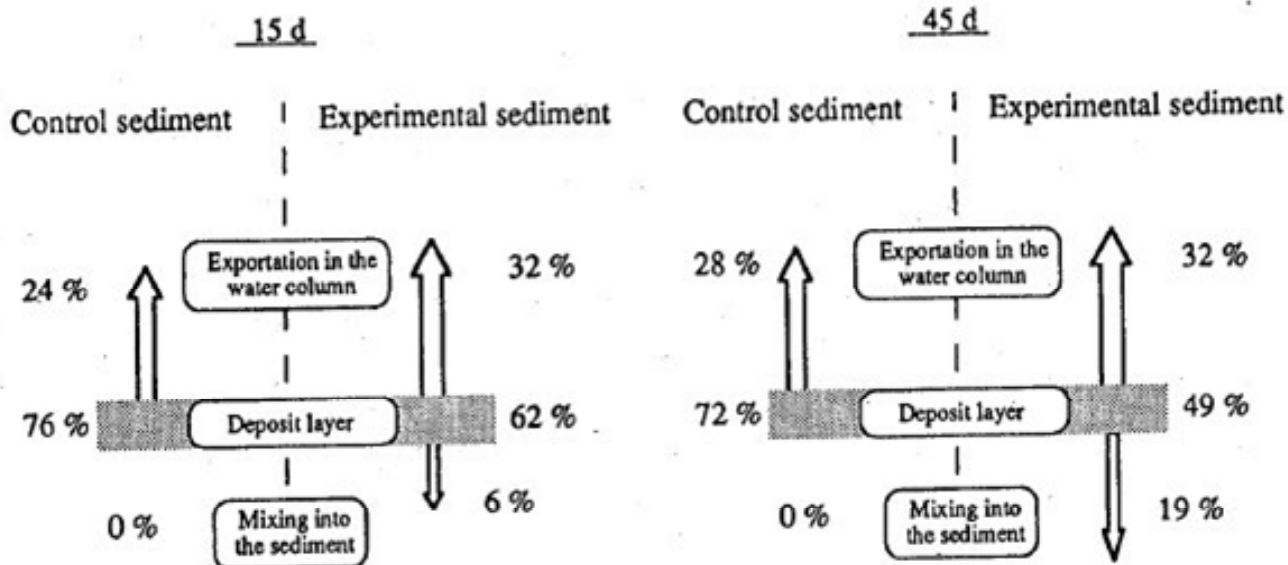


Figure 5: Luminophore transfers in non-bioturbated control sediment (left) and the experimental sediment with 6 *Nereis diversicolor* (right), after 15 and 45 d. Values are expressed in percentage of the initial luminophore input at the sediment surface.

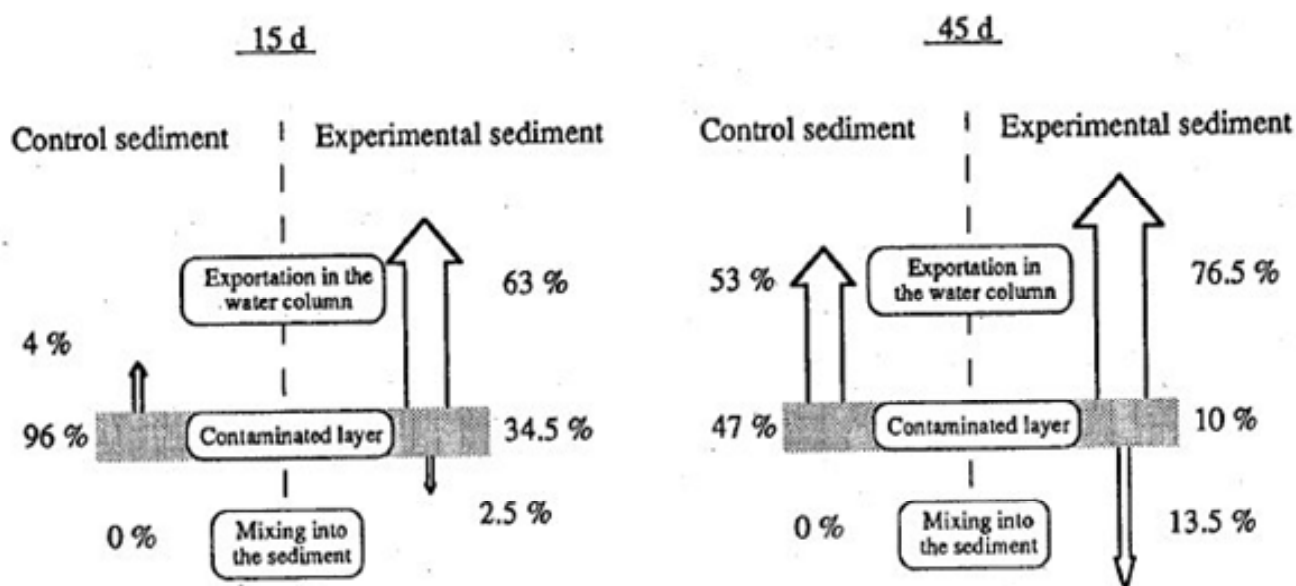


Figure 6: Hydrocarbon transfers in non-bioturbated control sediment (left) and the experimental sediment with 6 *Nereis diversicolor* (right), after 15 and 45 d. Values are expressed in percentage of the initial hydrocarbon input at the sediment surface.

During *in vitro* flow-through experiments, Gordon et al.²⁴ have found a 5-day removal of *n*-alkanes (from four API reference oils) averaging 72 % in presence of the polychaete *Arenicola marina*, and Mc Elroy et al.¹⁵ have shown a 2.4 increased release of PAH (polycyclic aromatic hydrocarbons) in sediment inhabited by *Nereis virens*, after 17 days.

The burial rate for hydrocarbons and luminophores appears to be very similar, 13.5 % and 19 %, respectively, after 45 d. Sediment reworking occurs during the building of burrows by worms, where superficial material is added to burrow walls and trapped by mucus. Luminophores and hydrocarbons adsorbed to sediment particles are subjected to the same process. In the absence of macrofauna, no burying occurs.

On the other hand, exportation in the water column differs in relation with the tracer. Whereas in the burying process, where it is the hydrocarbons adsorbed to sediment particles that are principally concerned, the exportation into the water column involves desorbed hydrocarbons. In contact with the polychaete burrows, hydrocarbons are diffused out of sediment pore water and are transported into the water column by both simple diffusion and the irrigation activity of the worms. Polychaete burrows increase the surface of sediment exposed to the water column and therefore the diffusion exchanges^{1,25}.

Biodegradation by microorganisms also appears to be responsible for some of the hydrocarbon removal observed. Among oil components, isoprenoid alkanes, such as pristane and phytane, are more stable and can serve at the corresponding boiling point as references to assess the degradation of the normal alkanes. Thus, the *n*C17/pristane and *n*C18/phytane are used as measures of microbial degradation of the normal alkanes²⁶. After 15 days, no biodegradation occurred in control and experimental sediments (data not shown). After 45 days, in the surface contaminated layer, *n*C17/pristane and *n*C18/phytane ratios remained of the same order as those of the "hydrocarbon cake" (figure 3), respectively of 5 and 3.5 (control sediment), and 5.1 and 2.7 (inhabited sediment). On the other hand, after 45 days, the *n*C17/pristane and *n*C18/phytane ratios calculated from SF of buried hydrocarbons decreased respectively to 2.3 and 1.1 (ratios are presented in figure 4) indicating microbial degradation of normal alkanes in polychaete burrows where microbial activities are enhanced²⁷.

Several authors have already reported the stimulation of degradation of added hydrocarbons in sediment in presence of polychaetes^{13,14,15}, but they used pure PAH mixtures that are more accessible for microorganisms than complex crude oil.

In conclusion, although the reworking activity of *Nereis diversicolor* is reduced in presence of Arabian Light crude oil, bioturbation by the polychaetes significantly influences the fate of hydrocarbons in sediment. During building of burrows, the polychaetes select particles from the sediment surface, resulting in a downward transport of surface sediment and adsorbed hydrocarbons. However, the major influence of polychaetes on hydrocarbons is their removal from sediment. Associated with the enhanced surface area from burrowing,

irrigation of burrows by *Nereis diversicolor* also contributes to the diffusion loss of desorbed hydrocarbons from the sediment. Normal alkanes are also degraded in burrows.

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